Occurrence of Sperm Abnormality of Beef Cattle at Several Artificial Insemination Centers in Indonesia

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Abstract . In the most species studied sperm abnormalities have long been associated with male infertility and sterility. This study evaluated the sperm morphology (normality and abnormality) of beef cattle at several Artificial Insemination centers in Indonesia. Total of 142 bulls were used in this study; an ejaculate from each bull was examined. A drop of semen was placed on 3-4 glass slides, and smears were prepared and air-dried. The smears were stained with carbolfluchsin-eosin (Williams stain). Types of morphological abnormalities were recorded from 500 cells on each sample. It was recorded that 77.46% samples had low primary sperm abnormalities (<5%), while the high level of primary sperm abnormalities (>10%) was found in 5.63% samples. Pear shaped was the most frequently type of abnormality found on examined samples (2.24 ± 2.94%); while double head was the lowest (0.01 ± 0.04%).

Key words : sperm abnormality, beef catlle, artificial insemination center

Introduction

Beef cattle are cattle raised for meat production. The popular breeds of beef cattle raised in Indonesia are Limousine, Simmental, Angus, Ongole, Brahman, and Bali. The major problem in beef cattle industry is the limited stock for beef cattle fattening. The establishments of Artificial Insemination (AI) centers are one of the government's efforts to overcome the problem. One of AI centers major tasks is to provide qualified bull frozen semen.

Bulls are selected based on their pedigree, or bulls which have passed the progeny test. Another method of bull selection is by using breeding soundness evaluation (BSE) or breeding soundness evaluation (BBSE) which is a technique to identify individual problems affecting the bull fertility (Allexander, 2008). This technique has been applied on rams (Bagley, 2009), boars (Sutkevičienė and Žilinskas 2004), stallions (Griffin, 2000), and bulls (Godfrey and Dodson, 2005; Makhzoomi et al*.,* 2007). In general BSE consists of three parts; physical evaluation (evaluation on external genital organs and rectal exploration), measurement of scrotum circumference and semen analysis. Semen analysis commonly included in BSE are sperm concentration, sperm motility and sperm morphology (Al-Makhzoomi et al*,*. 2008), mass activity (Fitzpatrick et al., 2002), and acrosome integrity (Hoflack et al*.,* 2006).

Assessments of sperm motility and sperm concentration have been well developed in Indonesia; several AI centers are equipped with computerized instruments. On the other hand, assessments on sperm morphology have not been done as much, although many studies had demonstrated the effects of abnormal sperm morphology to infertility (Chenoweth, 2005; Saacke, 2008). Different researchers and laboratories have a different determination on sperm abnormalities. Chenoweth (2005) classified the sperm abnormalities into two categories; first category classifies the sperm abnormalities into primary and secondary damages, while the second category classifies the sperm abnormalities into major and minor defects. Primary damage to the sperm occurs during spermatogenesis; while secondary damage occurs after spermiation. Classification of sperm abnormality into major and minor damages is based on the effects of the damage

on the male fertility. Major sperm damages greatly influence male fertility, while the minor sperm damages only slightly influence the fertility (Chenoweth, 2005). Ax et al. (2000) classified sperm abnormality into three groups: primary (those highly associated with sperm head and acrosome), secondary (those with a mid piece cytoplasmic droplet), and tertiary (tail damage).

Secondary and tertiary sperm damages on the tails are able to detect during evaluations on sperm motility or individual cellular movement; however, primary sperm abnormalities such as microcephalus, pear shaped, and narrow at the base, have a normal motility; therefore, the assessment of sperm morphology should be properly performed prior to insemination. Insemination with high sperm head abnormality > 10% can reduce the success rate of fertilization (Al-Makhzoomi et al*.,* 2008), which subsequently can decrease the success rate of artificial insemination (Sarder, 2004).

Considering the high numbers of AI centers in Indonesia and the shortage of skill to evaluate sperm abnormalities, this study was conducted to evaluate bull sperm morphology by focusing on the primary sperm abnormalities at several AI centers in Indonesia, and compare sperm abnormalities found in different breeds of beef cattle.

Materials and Methods

Semen sample. Semen sample collected from 14 AI centers in Indonesia, a total of 142 beef cattle bulls used in the study. Staining and evaluations of sperm abnormality were done at Reproductive Rehabilitation Unit Laboratory, Faculty of Veterinary Medicine, Bogor Agricultural University.

Sample Preparation. Fresh semen collections and smears preparations were performed from bull in AI centers, the samples were then delivered to the research Laboratory in Bogor. Sample collections were performed according to the standard protocol: a drop of semen was placed on the a glass slide, mixed with four drops of physiologic saline, homogenized by using a stick, and smeared on different glass slides. Smeared semen samples were air dried, coded according to the bull ID, and packed in a glass slides box.

Sample Staining. Smeared samples were stained with carbolfuchsin-eosin according to the method described by Williams in 1920 and modified by Lagerlof in 1934 (Kavak et al*.,* 2004). Steps in Williams staining protocol were as follow. The air-dried, thin smears of fresh semen on glass slides from AI centers were fixed in flame, washed with absolute alcohol for 4 minutes, and air dried. Then, the smears were treated with 0.5% chloramines solution for 2 minutes until the mucous disappeared and the smears looked fairly clear. The smears were washed in distilled water, rinsed in 95% alcohol, and stained with Williams for 8-10 minutes. Finally, the smears were washed in running water and allowed to dry.

Five hundred sperm were counted on each smear using a light microscope at 400x magnification (Olympus CH 20) sperm morphology was examined by evaluation at the head abnormalities. All types of sperm abnormality were recorded and classified. The classification of primary sperm abnormalities was based on Barth and Oko (1989) such as : a). Pear shaped sperm have their acrosomal (anterior) regions full of chromatin and rounded, while the post acrosomal region is narrow and slightly elongated, with a distinct margin between the anterior and posterior regions; b) Tapered head have their acrosomal regions bigger than normal sperm and tapers toward the post acrosomal regions; c) Narrow at the base often looks similar to pear shaped, however, on this type of sperm abnormality; the head appears elongated without a distinct margin; d) Narrow head are paddle-shaped sperm; this type of defects on the sperm head occurs in acrosomal region, with the post-acrosomal region is narrowed due to an incomplete development of primary spermatocyte; e) Sperm with abnormal contour include those possessing abnormal shapes of the heads and tails. f) Underdeveloped (undeveloped) sperm are those not adequately or normally developed; the sperm can be small, have short tails and incomplete cellular material perceived on further evaluation; g) Variable size is a term to describe sperm possessing head abnormalities

which are bigger (macrocephalus) or smaller (microcephalus) than normal size; h) Knobbed acrosome defect occurs in the acrosome region of sperm; the apex of the acrosome is flattened or indented; i) Double head sperm have two heads and a tail. The size of the two heads may varies; it can be similar, different, smaller, or bigger than normal size; j) Abaxial is a type of sperm abnormality whereas the implantation fossa of the tail is off-center. This type of abnormality often occurs along with another type of sperm abnormality, the accessory tail syndrome; k) Detached head occurs when the sperm head is separated from the tail. In this study, we classified the primary sperm abnormality rates into four categories; low (<5%), moderate (5-10%), high (10.1-15%), and very high (>15%).

Data Analysis

Data are reported as means ± SD Due to high levels of variability relative to the sample size, not all data subsets were normally distributed. Differences in ejaculate and breed were assessed using ANOVA and Fisher's Least Significant Difference tests for normally distributed data subsets, data were analyzed by analysis of variance procedures using Minitab software version 14.0.

Results and Discussions

In this study, most bulls were Simmental (49.30%); PO, Ongole, and Simbrah had the least numbers, consisted of only an animal on each breed (Table 1).

Primary sperm abnormalities evaluated on semen samples include pearshaped, narrow at the base, narrow (tapered head), abnormal contour, underdeveloped, round head, variable size (macrocephalus/microcephalus), double head, abaxial, knobbed acrosome defect, detached head, and diadem (Figure 1).

Table 1. Beef cattle breeds at 14 AI centers in Indonesia

Breed	Sample	Percentage (%)
Limousine	30	21.13
Simental	70	49.30
Brahman	12	8.45
Brangus	3	2.11
Angus	2	1.41
Bali	22	15.49
Simbrah	1	0.70
PO	1	0.70
Ongole	1	0.70
Total	142	100%

An important criterion in evaluating the quality of bovine semen is the morphology of the sperm cells (Barth and Oko, 1989). In fact, 10 to 25% of sperm, even in the ejaculates of highly fertile bulls, may be abnormally shaped (Bearden and Fuquay, 1997). In general, without considering the individual factors or the sperm abnormality types, sperm abnormality level of bulls at AI centers in Indonesia was low (3.2 ± 1.95%).

Figure 1. Bull sperm morpholoy stainend with carbolfuchsin seen thought a light microscope (Olympus CH20) 400 x magnification. Arrow a) Normal sperm b) Pearshaped c) Abnormal contour and d) Underdeveloped

Descriptively, the sperm abnormality levels were varied markedly between different breeds, without considering the numbers of bulls in every breed. The highest incidence on sperm abnormalities was found in Simmental-Brahman cross bulls (6.6%), while the lowest was found in Ongole (1.2%) and PO (1.2%). The highest incidence of sperm with pear shaped head among bulls occurred in Simbrah (5.4%) and Angus (4.9 ± 4.4%) (data not showed). Among all types of sperm abnormality, the pear shaped (pyriform) was at the highest incidence (2.24 ± 2.94%), some bulls normally produce

spermatozoa that are pyriform in shape and, as a consequence, have been suggested to be sub-fertile (Parkinson, 2004). The incidence of narrow at the base sperm abnormality was 0.32 ± 0.63%, narrow head sperm are 0.18 ± 0.28%, narrow head sperm lose their fertilizing capacity due to acrosomal damage (Barth and Oko, 1989). Sperm with abnormal contour and underdeveloped sperm are called teratoid sperm (Barth and Oko 1989), which includes sperm having such a severe aberration in structure and are incapable of fertilizing; this occurs due to primordial cell degeneration in seminiferous tubules. The incidence of sperm with abnormal contour and underdeveloped sperm is 0.14 ± 0.30% and 0.16 ± 0.36%, respectively. The results are similar with those reported by Barth and Oko (1989) that the incidence of these abnormalities was less than 1%. These abnormalities were believed to be genetic in origin, and not caused by accidents, diseases, or stress.

We found that microcephalus was at a higher incidence than macrocephalus acccording to Barth and Oko (1989) the incidence of microcephalus was less than 1%; also, macrocephalus abnormality of bull sperm was highly associated with the genetics. Both of these abnormalities occur due to the lack or excess of nuclear chromatin, contributing to the nuclear chromatin formation. The incidence of double head sperm was extremely low (0.01 ± 0.04%) as well as the incidence of abaxial sperm. This type of sperm abnormality was believed due to hereditary genetic defects (Barth and Oko, 1989) and also, genetics in origin (Chenoweth, 2005). The incidence of knobbed acrosome defect was 0.17 ± 0.45%, Thundathil et al. (2000) reported that no spermatozoon with a knobbed acrosome defect could penetrate the zona pelusida. The occurrences of detached head in this study were very low (0.02 ± 0.09%) this confirms report of Barth and Oko (1989) that detached head is usually found in small numbers (less than 10%) is commonly associated with testicular hypoplasia.

Among the breeds consisting of more than 10 bulls, the highest incidence of primary sperm abnormalities was found in Simmental bulls (4.8 ± 4.2%); this was significantly higher ($P<0.05$) than those in Bali bulls (1.6 ± 1.7%). There were not significantly different on the primary sperm abnormality levels between Simmental, Limousine, and Brahman bulls (Table 2).

Table 2. Primary sperm abnormality levels in 4 beef cattle breeds at AI centers

Breed	Percentage (%)
Simental	4.8 ± 4.2a
Limousine	3.6 ± 3.7ab
Brahman	2.6 ± 1.9ab
Bali	1.8 ± 1.65b

The highest incidence of primary sperm abnormality among 4 breed was pear shaped. It's found 2.9 ± 3.2; 2.1 ± 4.0; 1.4 ± 1.6 and 0.9 ± 1.1% in Simmental, Limousine, Brahman and Bali Bulls respectively (Table 3). In this study, from the total of 142 semen samples, 77.46% were in the low category rate of primary sperm abnormalities; while 16.90%, 3.52%, and 2.11% were in the moderate, high, and very high categories, respectively (Table 4).

Aberrations in sperm morphology were first describes by Williams in 1920 and Lagerlof in 1934 (Kavak et al., 2004) as a valuable aid in assessing the potential fertility of bulls. A high incidence of abnormal spermatozoa has since been confirmed to be associated with reduced fertility (Saacke, 2008; Sarder, 2004) and according to Padrik and Jaakma (2002) the level of sperm abnormality correlated with age of the bull, younger bull usually had higher sperm abnormality than older.

Unfortunately, the evaluation of sperm morphology is subject to great observer bias and requires careful technical training to obtain reliable assessments. The maximum abnormality rate allowed in fresh semen which will be processed into liquid or frozen semen is varied between technicians. Hoflack et al. (2006) and Alexander (2008) recommended that a bull had to have at least 70% sperm with normal morphology to pass the BSE test; in other words, maximum 30% of the sperm were allowed to have abnormalities. Balls and Peters (2004) demonstrated that a bull with more than 17% of its sperm abnormal wouldn't have a high fertilizing capacity. Ax et al. (2000) reported when sperm abnormalities occurred more than 20% in semen, the fertilizing

capacity was lower. The maximum acceptable sperm abnormality rate in semen to be processed into good frozen semen was not explicitly mentioned in 2005 INS. In reference to the INS standard which allows a maximum of 20% sperm abnormality rate including both primary and secondary abnormalities, it is expected that the primary sperm abnormality rate does not exceed 10%. Focus of this study was primary sperm abnormalities; this was based on the consideration that secondary sperm abnormalities affecting the tail could be self-selected during sperm motility examination. Sperm with tail abnormalities, such as coiled or bent tails automatically do not show a progressive movement. Secondary sperm abnormalities are usually due to environmental factors and are easy to fix. On the other hand, primary sperm abnormalities affecting the head cannot be detected during sperm motility evaluation; sperm with microcephalus, narrow head, or narrow at the base may have a more progressive movement than normal sperm because of their thinner shape.

Some primary sperm abnormalities are genetics in origin and hereditary. Primary sperm abnormalities, such as knobbed acrosome defect and round headed, as well as several tail abnormalities, such as mid piece defect, are genetics in origin and hereditary (Chenoweth, 2005). Mid piece defect is serious because it affects the location of mitochondria, which is responsible on the conversion of ATP and ADP to energy for the sperm movement (Silva and Gadella, 2006).

A raise question: what is the allowed percentage of primary sperm abnormalities? Since there were no report on the minimum or maximum percentage of primer sperm abnormality in cattle bull and considering of 20% maximum sperm abnormality rate allowed according Ax et al. (2000), including the primary and secondary sperm abnormalities, we assume that the primary sperm abnormalities should be less than 10%. Base on this research we found 8 bulls (5.63%) which did not have qualified semen for further processing into frozen semen (Table 4).

Further evaluations demonstrated that the primary sperm abnormality rates of some of these bulls were >23.6%, and if we added with an estimated of 10% secondary sperm abnormalities were also present in the sample, the semen total sperm abnormalities were more than 30%; this was way above the standard for frozen semen according to Ax et al. (2000). Based on these findings, evaluation of sperm morphology is an essential step; and serious attentions are needed on bulls with high levels of sperm abnormalities to avoid a decreasing quality of cattle, as well as the distribution of poor-quality semen. This fact also confirms that sperm morphology has to be asses individually.

Table 3 Primary sperm abnormality levels on different beef cattle breeds

Sperm Abnormality	Breed			
	Simmental	Limousine	Brahman	Bali
Pear shaped	2.9 ± 3.2	2.1 ± 4.0	1.4 ± 1.6	0.9 ± 1.1
Narrow at the base	0.5 ± 0.8	0.3 ± 0.4	0.1 ± 0.1	0.1 ± 0.2
Narrow	0.2 ± 0.4	0.1 ± 0.2	0.1 ± 0.2	0.2 ± 0.2
Abnormal contour	0.2 ± 0.3	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.5
Underdeveloped	0.2 ± 0.4	0.3 ± 0.5	0.0 ± 0.1	0.1 ± 0.1
Round head	0.1 ± 0.2	0.1 ± 0.3	0.0 ± 0.1	0.0 ± 0.0
Macrocephalus	0.0 ± 0.0	0.03 ± 0.1	0.1 ± 0.2	0.0 ± 0.1
Microcephalus	0.2 ± 0.9	0.1 ± 0.2	0.1 ± 0.1	0.1 ± 0.1
Double head	0.0 ± 0.0	0.0 ± 0.0	0 ± 0.0	0.0 ± 0.0
Abaxial	0.1 ± 0.2	0.1 ± 0.2	0.3 ± 0.4	0.2 ± 0.3
Knnobbed acrosome defect	0.1 ± 0.3	0.2 ± 0.9	0.15 ± 0.2	0.2 ± 0.2
Detached head	0.0 ± 0.1	0.0 ± 0.1	0 ± 0.0	0 ± 0.0
Diadem	0.2 ± 0.3	0.1 ± 0.3	0.2 ± 0.4	0.1 ± 0.2

Table 4. Classification on the primary sperm abnormality levels of bulls in Indonesia

Category	Abnormality level (%)	Number of bulls	Percentage
Low	0 – 5	110	77.46
Moderate	5.1 – 10	24	16.90
High	10.1 – 15	5	3.52
Very high	>15	3	2.11
Total		142	100.00

Conclusions

In general, this study concluded that the primary sperm abnormality levels of bulls at AI centers in Indonesia were low; and there were around 5.63% bulls did not have qualified semen for further processing to be frozen semen. We recommended all bulls at AI centers in Indonesia have to pass sperm morphology evaluations, so that in the future, the distribution of bull semen with high levels of sperm abnormalities is avoided.

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